PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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To:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/000873	07.03.2005	10.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N9/72, C12N15/58, C12N15/62, C12Q1/37

Applicant
ASTRAZENECA AB

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2005/000873

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26, 28-31
	No:	Claims	27, 32
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-32
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 CITED DOCUMENTS

Reference is made to the following documents:

D1: ZESLAWSKA E ET AL: "Crystals of the urokinase type plasminogen activator variant betac-uPA in complex with small molecule inhibitors open the way towards structure-based drug design" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 301, no. 2, 11 August 2000, pages 465-475

D2: TANG W ET AL: "An Efficient System for Production of Recombinant Urokinase-Type Plasminogen Activator" PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 11, no. 3, December 1997, pages 279-283

D3: WO 01/55174 A (OKLAHOMA MEDICAL RESEARCH FOUNDATION), 2 August 2001

D4: HANSEN A P ET AL: "Solution structure of the amino-terminal fragment of urokinase-type plasminogen activator." BIOCHEMISTRY. 26 APR 1994, vol. 33, no. 16, 26 April 1994, pages 4847-4864

D5: HAJDUK P J ET AL: "Identification of novel inhibitors of urokinase via NMR-based screening." JOURNAL OF MEDICINAL CHEMISTRY. 19 OCT 2000, vol. 43, no. 21, 19 October 2000, pages 3862-3866

2 NOVELTY (ARTICLE 33(2) PCT)

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 27 and 32 is not new in the sense of Article 33(2) PCT.

2.1 Document D1 discloses the expression of a C122S mutant of the uPA catalytic domain in *E. coli*, recovering of the protein from inclusion bodies by denaturation with 6M guanidinium chloride, refolding with a buffer comprising 4 mM reduced glutathione and 0.02 mM oxidised glutathione (ratio 200:1) at pH 8.0 and activation with plasmin. The document further discloses the crystallisation of C122S βc-uPA

and analysis by x-ray crystallography (cf. page 474, left column).

2.2 As a product is not rendered novel by the fact that it is produced by means of a new process, the uPA described in claim 27 can not be distinguished from the above prior art. Claims 27 and 32 are therefore not new (Article 33(2) PCT).

2.3 Claims 1-26 and 28-31 however appear to be novel, as none of the documents cited discloses the solubilization of a uPA variant from inclusion bodies with a buffer at pH 8.5-10.5, the buffer comprising a redox pair, whereby the reducing agent is present in excess, the concentration of the reducing agent being at least 5 mM. Moreover, a uniformly ^{15}N labelled soluble uPA variant and the identification of ligands for uPA by NMR analysis, isothermal titration calorimetry or differential scanning calorimetry are not disclosed in the prior art cited.

3 INVENTIVE STEP (ARTICLE 33(3) PCT)

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-32 does not involve an inventive step in the sense of Article 33(3) PCT.

3.1 Document D1 represents the most relevant state of the art and discloses the preparation of a soluble, active, human uPA variant (C122S βc-uPA) from inclusion bodies using a buffer with a pH of 8.0, comprising 4 mM reduced glutathione and 0.02 mM oxidised glutathione (ratio 200:1) (cf. page 474, left column).

3.2 The subject-matter of claims 1-26 differs in the pH-value and the composition of the refolding buffer, e.g. the concentration of the reducing agent, or the ratio of reducing agent to oxidising agent. The problem to be solved may therefore be regarded as providing a further buffer for refolding uPA from inclusion bodies.

3.3 The solution proposed in claims 1-26 can not be considered inventive (Article 33(3) PCT) as it is a customary practice for the skilled person to modify/ adapt an experimental protocol by slightly changing the pH-value and concentration of the composition of a buffer, which does not require inventive skills. In particular, since several protocols for the preparation of uPA from inclusion bodies, i.e. several

refolding buffer, are already described in the prior art (cf. documents D1, D2 and D3).

3.4 The same comment also applies to claims 28-31, which refer to soluble uPA variants prepared from inclusion bodies and embrace obvious embodiments such as a uniformly isotopically labelled uPA and the identification of ligands of uPA by NMR analysis, isothermal titration calorimetry or differential scanning calorimetry. The provision of said means and methods is a routine laboratory practice for the skilled person and does not require inventive skills. Moreover, the uniform isotope labelling of the amino-terminal fragment of uPA and the identification of inhibitors of uPA by NMR-based screening have already been described in the prior art (cf. documents D4 and D5). The subject-matter of claims 28-31 is thus not inventive (Article 33(3) PCT).

4 ARTICLE 6 PCT

It is clear from the description, in particular from the examples, that the application relates to the preparation of soluble uPA from inclusion bodies and/or the refolding of uPA from inclusion bodies (cf. page 6, lines 20-23; example 1). The feature "inclusion bodies" is thus considered essential to the invention.

Since independent claim 1 does not contain this essential feature, it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.